# BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.**

**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : B03C 5/00 | A1

(11) International Publication Number: WO 98/04355

(43) International Publication Date: 5 February 1998 (05.02.98)

(21) International Application Number: PCT/GB97/02011

(22) International Filing Date: 28 July 1997 (28.07.97)

(30) Priority Data:
9615775.5 26 July 1996 (26.07.96) GB

(71) Applicant *(for all designated States except US)*: BRITISH TECHNOLOGY GROUP LIMITED [GB/GB]; 101 Newington Causeway, London SE1 6BU (GB).

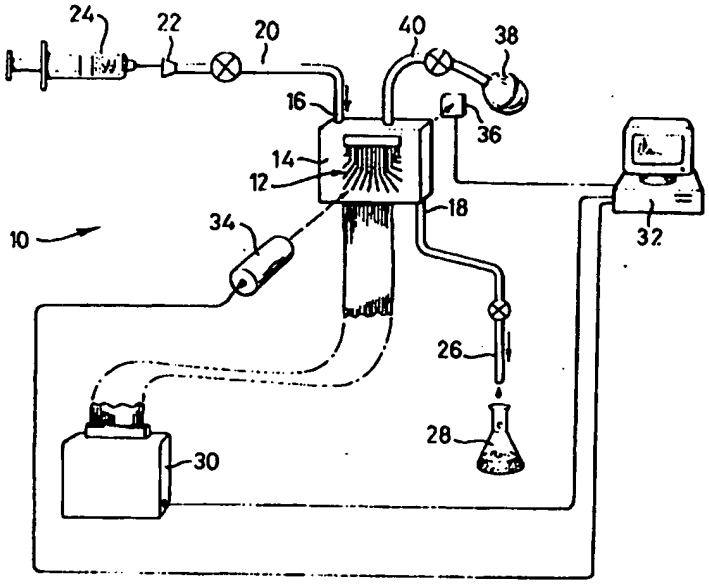
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: PETHIG, Ronald [GB/GB]; Lleyn, Telford Road, Menai Bridge, Gwynedd LL59 5DT (GB). MARKX, Gerardus, Hendricus [NL/GB]; 18 Hadfield Place, Glossop, Derbyshire SK13 8JE (GB).

(74) Agent: BUTTRICK, Richard; British Technology Group Limited, 101 Newington Causeway, London SE1 6BU (GB).

(81) Designated States: CA, IL, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*

(54) Title: APPARATUS AND METHOD FOR TESTING PARTICLES USING DIELECTROPHORESIS




(57) Abstract

The behaviour of particles is tested by placing a suspension of the particles in a chamber in which an electrode array is arranged to generate a spectrum of different frequency dielectrophoretic fields. The behaviour of the particles at the different frequencies can be studied in a convenient and rapid manner. The apparatus and method can be used for a variety of purposes, including characterising the properties of specified particle types, or analysing the particle population in a fluid. It is also possible to expose the particles to different fluid parameters to extend the test spectrum in other senses.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

# APPARATUS AND METHOD FOR TESTING PARTICLES USING DIELECTROPHORESIS

This invention relates to an apparatus and method for testing or investigating particles present in a fluid using dielectrophoresis, for example to determine the dielectrophoretic characteristics, or to identify the presence and/or relative concentration of a particular type or types of particle in the fluid.

Dielectrophoresis (DEP) is the translational motion of a particle caused by polarisation effects in a non-uniform electric field. Unlike electrophoresis, no overall electrical charge on the particle is necessary for DEP to occur. Instead, the phenomenon depends on the magnitude and temporal response of an electric dipole moment induced in the particle, and on the force produced as a consequence of the electric field gradient acting across the particle. The magnitude of the dielectrophoretic force $F_{dep}$ on a spherical particle of radius $a$ is given by:

$$F_{dep} = 2\pi a^3 \varepsilon_m Re\left[\frac{(\varepsilon_p^* - \varepsilon_m^*)}{(\varepsilon_p^* + 2\varepsilon_m^*)}\right]\nabla \bar{E}^2 \qquad (1)$$

where $\varepsilon_m$ is the absolute permittivity of the suspending medium, $\nabla \bar{E}$ signifies the gradient in the electric field, and $\varepsilon_p^*$ and $\varepsilon_m^*$ are complex permittivities of the particle and

its surrounding medium, respectively. The complex permittivity is given by $\varepsilon^* = \varepsilon - j\sigma/\omega$, where $\varepsilon$ is the absolute permittivity, $\sigma$ is the electrical conductivity, $\omega$ is the angular frequency of the electric field and $j = \sqrt{-1}$. The term Re indicates that the real part of the expression within the square brackets of equation (1) is to be taken.

For particles suspended in a uniform aqueous electrolyte, the permittivity and conductivity of the suspending medium usually remains approximately constant over the frequency range 100Hz to 100MHz, whereas for the particles themselves these parameters can vary significantly. The term $(\varepsilon_p^* - \varepsilon_m^*)$ can therefore be positive or negative, and thus over an extended frequency range a particle can exhibit both positive DEP (movement towards areas of high field strength) and negative DEP (movement towards areas of low field strength).

Differences in the dielectrophoretic frequency response of particles can be used to selectively separate them by dielectrophoresis. An example of a particle separator which operates on this principle is described in International Patent Application WO-A1-9422583 in which a fluid containing two types of particles flows over electrodes producing a non-uniform electric field which is controlled so that the two types of particle experience different resultant forces and the fluid flow can remove one particle type preferentially. This separator can thus separate dielectrophoretically different particles or cells, but to be used effectively the dielectrophoretic

behaviour of the two different particle types should be already known.

Pin-plate electrodes have been used for this purpose to determine the dielectrophoretic characteristics of particular particle types, but the procedures are laborious and time consuming.

Gascoyne *et al* Meas. Sci. Technol. 3 (1992) at pages 439 to 445, determines the DEP behaviour of 200-300 particles, specifically mammalian cells, by automatic image analysis. However, as with the use of pin-plate electrodes the DEP response of the cells can only be measured at a single frequency at a time. Because the frequency range of interest in DEP is relatively large (typically 100 Hz to above 10 MHz), and several data points per decade may be required, many single-frequency experiments need to be obtained for a sufficiently wide spectrum. Using this method it is therefore cumbersome and time-consuming to obtain a dielectrophoretic spectrum over a large frequency range, so as to facilitate the observation or manipulation of cells or particles.

There is therefore a need for a better way of determining the characteristics of particles in a fluid and/or of identifying one or more particle types present.

According to the present invention there is provided apparatus for testing particles present in a fluid comprising a chamber, a series of spaced electrodes in the chamber, means for applying electrical inputs of different frequencies to the respective electrodes to generate

different dielectrophoretic fields in respective regions adjacent the electrodes, and means for detecting the presence of particles in the respective regions.

Preferably means are also provided for varying other parameters in the chamber which can affect the dielectrophoretic response of the particles. Such parameters can include the electrical conductivity and/or permittivity of the material in the chamber and/or its pH value. Preferably also means are provided for adjusting the voltage of the electrical inputs to the electrodes.

Additionally or alternatively, other forces may be used to enhance the movement of the particles. These may include hydrodynamic, ultrasonic, electrophoretic or optical forces.

The apparatus may be operated, for example, to determine the parameters which are appropriate for the separation and/or identification of a particular particle type in the fluid, or to differentiate between two particular types of particle present or to analyse a mixture of several particle types.

The regions in which the particles are to be detected will depend upon the geometrical configuration of the apparatus and the conditions at which it is operated. In one form of the invention, the electrodes are directed towards a further electrode or electrodes at a common or ground potential and the main areas of interest will lie in the spaces between the tips of the series of electrodes and the common electrode. Additionally or alternatively, the

regions between adjacent electrodes of the series are to be investigated.

The means to detect the presence of particles in each of said regions may comprise a source of electromagnetic radiation which is transmitted through the chamber to impinge upon particles present in the electrode gaps, and sensing means to detect the transmitted radiation not absorbed by said particles. There may be respective radiation sources for each region of interest adjacent the series of electrodes, or beam deflecting means may direct the radiation for a single source through the regions successively.

The electromagnetic radiation source may be a laser and the detector may be a charge coupled device (CCD). Alternatively, a video camera can be provided to monitor the radiation transmitted and a light source other than a laser can be employed. Automated image analysis means can then be used to interpret images thus obtained.

Other means to detect the presence of particles may include current and/or voltage sensing circuits, connected in series with each of said electrodes, and arranged so as to detect variations in field characteristics and/or impedance fluctuations within the electrode gaps. The information may then be used to indicate the presence of particles adjacent to the electrodes. Automatic electronic switch means may be provided for switching such sensing circuits between the electrodes. This may be effected sequentially.

Any of these detection techniques may additionally employ means for obtaining information about the temporal dielectrophoretic response, that is to say, the speed at which particles move to or from different dielectrophoretic field regions. Because the speed of movement of the particles is directly related to the forces acting on them, and because those forces are also related to the field characteristics, such temporal information (eg. rate of arrival of particles) may help to corroborate other measurements or may be used independently to identify and/or characterise particles.

The series of electrodes may be configured as a series of elongate fingers in a comb-like array with their tips directed towards a common electrode in the form of a linear conductive strip disposed opposite the array. In a modified configuration, the electrode array is arranged in a radiating pattern, for example of a circular or part-circular form. In one such arrangement, the electrodes are disposed about the periphery of a disc-shaped support such that their distal ends point towards a central region where the common electrode is situated. In the case of a circular electrode array the common electrode will be disposed centrally. In another example the electrodes may radiate outwards to point towards a peripheral common electrode and the chamber may be of any suitable shape and dimension to accommodate the electrode array.

The electrodes may be applied to the surface of a non-conducting substrate, such as glass or silicon, by

conventional techniques used in the semi-conductor industry to apply conductive tracks. Alternatively, electrically conductive electrodes may be applied, eg. by screen printing, onto a porous membrane. The use of porous membranes may have other functions, such as for the removal or capture of larger particles. The porous membranes may also be used to move particles towards or away from the electrodes, or be used to help establish a conductivity or permittivity or pH gradient or other gradient within the chamber.

A separate fluid supply means may be connected to the chamber so as to supply additional fluid for flushing particles through the chamber and/or cleaning the chamber and/or modifying the overall conductivity and/or pH of the contents of the chamber.

According to another aspect of the invention there is provided a method of testing particles comprising locating the particles in a carrier fluid in a space in different regions of which they are subjected to a plurality of different dielectrophoretic fields and detecting the presence of the particles in the respective regions in order to characterise or identify the particles detected.

The method may be employed with all types of particle, including animate and inanimate biological particles such as cells, and other kinds of organic particle as well as particles of inorganic matter.

Solely by way of example, the method and

apparatus of the invention will now be described in more detail with reference to the accompanying drawings in which:

Fig. 1 is a schematic illustration of one form of apparatus according to the invention;

Fig. 2 is a larger scale plan view of a multi-electrode array used in the apparatus of Fig. 1;

Fig. 3 is a block diagram showing the signal generator of Fig. 1 in more detail;

Figs. 4a, 4b and 4c are graphs showing the number of cells collected in the use of the apparatus of Fig. 1 under different conditions, and

Figs. 5 and 6 are schematic illustrations of two modified forms of apparatus according to the invention.

Fig. 1 shows an apparatus 10 for testing or characterising biological cells using dielectrophoresis. A multi-electrode array 12 housed within a chamber 14, consists of a comb-like series of spaced electrodes, shown in more detail in Fig. 2, the tips of which extend close to a common ground electrode 13. Connected to an inlet 16 of the chamber is one end of a synthetic plastics or a rubber tube 20. A syringe 24 is connected, through a bung 22, to the other end of the tube 20. The syringe initially contains a carrier liquid in which the cells to be studied are suspended. Through a second tube 26 connected to outlet 18 of the chamber 14, fluid can be drained from the chamber into a flask 28. The individual electrodes of the electrode array 12 are connected to a signal generator 30 which is operated under the control of a micro-computer 32.

A laser 34, which is also under the control of micro-computer 32, is arranged to direct a beam through the chamber 14. A detector 36 sensitive to the wavelength of the laser beam, such as a charged coupled device (CCD) or a similar photosensitive device, is positioned on the opposite side of the chamber to the laser 34. The micro-computer 32 is also programmed to store and process the signals obtained by the detector 36. Optionally, pump 38 can deliver fluid to the chamber via tube 40.

In operation, the cell suspension is introduced from the syringe 24 via tube 20, or pump 38 via tube 40, into chamber 14. Under control of micro-computer 32 the signal generator 30 activates the electrodes in the multi-electrode array 12 simultaneously at different frequencies so that a series of different dielectrophoretic fields are established, in particular between the tips of the individual electrodes and the common electrode 13. Attraction or repulsion forces experienced by individual cells in these fields can urge the cells preferentially towards or repel them from different dielectrophoretic regions. The liquid may be at rest while the dielectrophoretic fields are established and the cells are distributed in accordance with the forces they experience from the fields, or a closed circulatory flow can be established, so that the particles will continue to be exposed to different field forces unless they have been captured by a force gradient in any particular region.

The signal generator provides a spectrum of

different frequency fields, eg. from 100Hz to 10MHz, in the chamber 14. By establishing the different frequency dielectrophoretic fields in different regions of the chamber, it is possible to observe any tendency the cells of any specific cell type have to accumulate close to the tips of particular electrodes, due to being subjected to repulsion and/or attraction forces by the fields at the different regions. Under the control of the micro-computer 32, the laser 34 illuminates the regions of the different fields sequentially. The radiation transmitted to the CCD 36 will be obscured to a greater or lesser extent, depending upon the amount of cells in each region so that the CCD 36 detects different radiation intensities in accordance with the amount of cells which have accumulated around particular electrodes. It is of course possible to obtain measurements simultaneously from the regions of the different fields by using an array of lasers and associated detectors.

Digital signals of the radiation intensities obtained are indicative of the amount of absorption at each of the regions of the multi-electrode array and are stored in the micro-computer 32, together with information, from the signal generator 30, about the frequencies of the fields at these absorption regions. Also stored in the micro-computer are data on other parameters which may influence the behaviour of the particles in the dielectrophoretic fields and so be able to be used to identify and/or characterise the particles. For example,

the conditions in the carrier fluid in the chamber, such as the conductivity and pH may be relevant. This data may be entered manually from initial measurements or may be monitored during operation. The information gathered may, for example, be compared with a look-up table, stored within the micro-computer 32, to derive information about the identity of the cells.

Other conventional counting methods may be used, including automatic image analysis, or making optical density measurements. It will be appreciated that using image enhancement algorithms, computerised images can be obtained, stored and analysed.

From a series of tests a database of spectra of different cells and/or mixtures can be established or expanded to enable rapid identification of cells.

One form of electrode array 12 is shown in more detail in Fig. 2. It is fabricated using photo-lithography and comprises twenty gold plated conductors 12a,b,c...12t which provide a series of parallel electrodes 42 each 21$\mu$m wide and spaced apart a similar distance. The tapered tips of the electrodes extend close to the common ground electrode 13 and at their other ends splayed tracks 44 continue from the electrodes to broad area pads (not shown) at which the external electrical connections are made.

Fig. 3 shows the signal generator 30 in more detail and its connections to the electrode array. The signal generator comprises crystal oscillators 52,54 operating at frequencies of 10MHz and 1MHz respectively.

From the primary frequency outputs of the oscillators, lower frequencies are obtained using decade and binary counters, which may consist of 74-series decade counter TTL devices 74LS90. A first group of three counters 56, only two of which are illustrated, are connected in cascade to operate as divide-by-ten devices. The frequency oscillators 52,54 and the counters 56 are each connected to the further group of five counters 58, only four of which are illustrated, which operate as divide-by-two counters to provide a series of outputs of frequencies in the ratios of 0.5, 0.25 and 0.125 to their input frequency. The generator thus obtains frequencies of 10, 5, 2.5, 1.25 and 1MHz, 500, 250, 125, 100, 50, 25, 12.5, 10, 5, 2.5, 1.25 and 1kHz, and 500, 250 and 125Hz. Through voltage control amplifiers 60, the 0-5V square-wave signals from the counters are converted to square-wave signals of ±5V which are supplied to the electrodes of the array 12.

A complete dielectrophoresis spectrum of a particle suspension can thus be obtained in a single experiment by applying signals of equal voltage but different frequency to respective electrodes in the multi-electrode array. Voltages in the range 0-24V pk-pk could be produced, as determined by the computer control, but typically voltages of between 2 and 5V pk-pk were employed in the experiments.

A specific example of the use of the apparatus of Figs. 1 to 3 with reference to experimental testing carried out by the inventors now follows.

The chamber 14 is rectangular and has a volume of 50μL. The electrode array 12 is formed on one wall and the internal space of the chamber is built up above the electrodes by using a 200μm polytetrafluoroethylene (PTFE) spacer disposed between and sealed to the opposite walls using epoxy resin as a water seal. An aqueous suspension of cells, having a predetermined electrical conductivity, was pumped (using a Gilson Minipuls 3 system) into and out of the chamber via 1mm inner bore polyvinyl chloride (PVC) and silicone tubing 16 and 18.

The particles used were yeast cells *Saccharomyces cerevisiae* strain RXII. The yeast was grown overnight at 30°C in a medium of pH 5 consisting of 5 g/L yeast extract (Oxoid), 5 g/L bacterial peptone (Oxoid) and 50 g/L sucrose, harvested and washed four times in deionised water. The suspension liquid also contained non-viable yeast cells obtained by heat treatment for 20 min at 90°C, and washed four times in deionized water. The optical density at 635nm of the final suspensions used was of the order of 0.3-0.4 in a cuvette of 1cm path length, corresponding to concentrations of the order 7-9 x $10^6$ cells/ml.

The multi-electrode array 12 was monitored under a microscope (not shown) coupled to a video camera and monitor having a CCD 36. After introducing a cell suspension into the chamber, the fluid flow was stopped and the electrodes 12 energised. Cells were observed to move directly to nearby electrodes, and also to migrate from

some areas in the electrode array towards other areas. After equilibrium conditions were established, which took of the order 10 seconds or less, the distribution of cells over the multi-electrode array 12 and in the region between the electrode 12 tips and the common electrode 13 were video recorded. Cell counts were then made in the areas between the electrodes and the area near the tips of the electrodes from the images captured by the CCD 36.

In a suspending medium of conductivity around 0.6 mS/m, viable yeast cells moved from electrodes energised at frequencies around 10 kHz and below, and were attracted towards those operating at 50kHz and higher. Non-viable yeast cells however were repelled from the electrodes operating at the frequencies of 1MHz and above, and collected around those energised at frequencies of 500kHz and lower. The resulting distribution is shown in the graph in Fig. 4a, and corresponds to the combined effect of cells moving directly to nearby electrodes under positive DEP, as well as to cells moving from areas of negative DEP to positive DEP.

Figs. 4b and 4c show the distribution of cells with the suspending fluid treated to change its conductivity by the addition of small amounts of a concentrated NaCl solution. The resulting conductivity of the suspending medium was measured with a HP4192A impedance analyser using platinum-black electrodes of cell constant $1.58cm^{-1}$.

Fig. 4b shows the results of a test, using only

viable yeast cells, in a medium of conductivity around 6 mS/m. The collection spectrum of the cells exhibits a strong peak around 0.1MHz to 1MHz and falls off to nil by 1kHz. From a further similar test, Fig. 4c shows the collection spectrum of non-viable yeast cells in a medium of conductivity around 0.45 mS/m and, although a degree of experimental scatter is present, it shows the concentration of the cells at lower frequencies, falling off almost to zero at 1MHz.

Characteristic frequency profiles can be established for different types of particles. From such data and particle counts at appropriate frequencies for the particle types present in a mixture it is possible to establish the relative concentrations of mixtures of known particles in a fluid medium.

In a time following these cell displacements in the tests described, other less dominant cell motions were observed. These motions took the form of cells appearing to stream from the common electrode and across the electrodes energised at the lower frequencies, to finally settle on the electrodes energised at frequencies in the range from around 5kHz to 500kHz. This phenomenon might be due to low-frequency electrophoresis effects. However, non-viable cells were also sometimes observed to flow from the common electrode towards the electrodes energised at the MHz frequencies, and so other DEP driven forces may also be responsible. With increasing time these additional cell migrations distorted the DEP collection spectrum

because they led to an over-estimate of positive DEP effects. This problem was overcome by gently pumping the cell suspension over the electrode array to agitate it. The induced hydrodynamic forces assisted a more even distribution of the cells in each frequency region, removed non-attracted cells, and reduced the convection-like streaming of cells without interfering with the DEP effects. If the flow rate became too high, cells that were only held by relatively small DEP forces were removed from the electrodes, and thus gave rise to a reduced estimate of the cell number at these electrodes.

The illustrative experiments show measurements of the DEP responses measured with constant medium conductivity and permittivity. It is within the scope of the invention to produce conductivity and/or permittivity gradients over the electrode arrays so that further DEP spectra can be obtained in a single run of cells or mixtures at various conductivities and permittivities and or pH values. Fig. 5 illustrates a modification of the apparatus in Fig. 1, in which the particle-containing fluid is divided between two containers 62,64 and the conductivity increased in the container 62. When pump 66 drives liquid to the chamber, the conductivity of the liquid in the chamber will increase progressively as liquid from the chamber 62 is drawn into and mixes with the liquid already in the chamber 64. In a similar manner, a pH gradient in the carrier fluid could also reveal, at the lower frequencies, effects associated with changes in the

surface charge of a cell. Such images could also be used for bioparticle characterisation and identification, of use for example in the clinical identification of micro-organisms.

In another development of the techniques described above, by placing an extended number of such multi-electrode arrays 12 in a conductivity gradient, permittivity gradient or pH gradient, for example, the dielectrophoretic frequency spectrum as a function of that parameter can also be obtained in a single experiment.

Fig. 6 illustrates schematically an apparatus of this form. An elongate chamber 70 has a series of electrode arrays 72a,72b,72c,72d set at intervals along its length. These arrays are indicated purely diagrammatically but may each take the form of the array already described with reference to Fig. 2. Inlet and outlet porting 74,76 respectively, at opposite ends of the chamber 70 are provided for the suspension of particles to be tested. Both the inlet and outlet porting are preferably arranged so as to give a relatively uniform velocity flow across the width of the chamber, eg. comprising a series of ports spaced across the width of the chamber. In the zone of each electrode array 72 along the length of the chamber 70, groups of inlet and outlet ports 78,80 are provided for passing a cross-flow of a further fluid over the array. For each array, a fluid having a different conductivity is used for the cross-flow, for example, the conductivity being progressively greater for each successive array

72a,72b,72c,72d.

In operation, the material under investigation is introduced into the chamber through the porting 74 and the electrode arrays are energised. Fluid flows are then directed through the cross-flow ports 78,80, over the arrays, each successive array 72a,72b,72c,72d being exposed to a medium of greater conductivity than its preceding array. A particle count is then performed at each array by means (not shown) which can take any of the forms mentioned earlier herein. If the particles are less strongly attracted by the dielectrophoretic forces as the conductivity of the medium increases, the particle count will be reduced at each successive array and a spectrum of values can be obtained from the different arrays. When the required data has been collected of the particle count, the cross flows are terminated and the debris is cleared by a flushing flow along through the ports 78,80.

It will be understood without further illustration that the method described can be employed to test dielectrophoretic behaviour with variations of other parameters, such as the permittivity or pH value of the surrounding fluid medium. Where appropriate, similar experiments can be run in which the conductivity or other variable parameter is reduced in the direction of main fluid flow through the chamber. Added versatility and applications can be achieved by coating the electrode arrays with reactive chemicals.

A wide variety of particles and non-biological

cells may be studied by the use of the invention, employing suitable electrode arrays and test parameters. For example, the order of magnitude of the gap between the series of electrodes and the common electrode could be altered so as to accommodate and test different bioparticles species such as viruses, prions, proteins, molecules or DNA, or chemically activated particles such as coated latex beads.

The fields of use of the invention include the dielectrophoretic characterisation of a presumed dominant, single-type, particle (animate or inanimate) suspended in an aqueous medium or other fluid, such as may be required for the inspection of liquefied food products, biological fluids such as urine or plasma, or of liquids sampled during a chemical production process. The method described provide rapid means for ascertaining the most appropriate conductivity value of the fluid and voltage frequency range to be used in the dielectrophoretic separation of a dominant particle type from the fluid, for example, the conductivity and frequency values required to obtain separation using positive or negative dielectrophoresis.

The procedure could be repeated on samples that had already been processed through a dielectrophoresis separation stage, so as to further characterise a separated particle or ascertain the experimental conditions required to separate other particle types which might have been present in the original sample.

Another area of application would be in the

dielectrophoretic characterisation of fluid samples, which should have a relatively homogeneous population of particles. Examples here include the monitoring of yeast cells in fermenting beer or wine, or of the lactic acid bacteria used as starter colonies in the fermentation of yoghurt or cheese, or of crystalites formed in a chemical production process. In these cases a rapid means would be provided for checking the presence, viability and homogeneity of the particle type. For example, the relative compositions of dead and live yeast, or of the starter organisms in yoghurt (typically streptococci and lactobacilli) would be given by the dielectrophoretic spectra produced as a function of conductivity, as well as an indication of the presence of spoiling impurities (eg. of yeast in yoghurt or of lactic acid bacteria in beer). The homogeneity (size and chemical composition) of crystallites sampled during a chemical process could also be monitored.

The invention could also be employed for the dielectrophoretic analysis of fluids containing several particle types. Examples here would include biological fluids such as urine, where the relative composition of Gram-positive and Gram-negative bacteria could be ascertained by obtaining dielectrophoretic spectra over a range of conductivity and pH values, for example to identify the presence of a dominant infective organism.

CLAIMS

1. Apparatus for testing particles present in a fluid comprising a chamber, a series of spaced electrodes in the chamber, means for applying electrical inputs of different frequencies to the respective electrodes to generate different dielectrophoretic fields in respective regions adjacent the electrodes, and means for detecting the presence of particles in the respective regions.

2. Apparatus according to claim 1 wherein the detection means comprises at least one electromagnetic radiation source arranged to direct radiation through said regions to impinge on the particles and at least one sensing means to thereby provide a signal for each said region indicative of the presence of particles in said region.

3. Apparatus according to claim 1 wherein the detection means comprises sensing means for measuring variations in the electrical characteristics in said regions.

4. Apparatus according to claim 1 wherein said sensing means are connected to the electrodes serially for said measurements, eg. of current and/or voltage.

5. Apparatus according to any one of the preceding

claims wherein the detection means comprise means for obtaining time-dependent data indicative of the rate and/or quantum of displacement of particles per unit time.

6. Apparatus according to any one of the preceding claims wherein means are provided to vary a parameter of the fluid carrying the particles progressively during the operation of the apparatus.

7. Apparatus according to any one of the preceding claims wherein the series of electrodes project towards a common conductive member.

8. Apparatus according to any one of the preceding claims wherein the electrodes are arranged as a bank of laterally spaced elongate elements.

9. Apparatus according to claim 7 and claim 8 wherein the electrodes are arranged in a radiating pattern of elongate elements.

10. Apparatus according to claim 9 wherein the electrodes radiate inwardly or outwardly towards a common electrode separated from the electrodes.

11. Apparatus according to any one of the preceding claims comprising means for varying a parameter of the fluid in the chamber while exposing the particles therein

to the dielectrophoretic fields, whereby to test the reaction of the particles to said variation.

12. Apparatus according to any one of claims 1 to 10 comprising a plurality of said series of spaced electrodes arranged in spaced zones of the chamber and means for supplying fluids having different parameters to the respective zones and for detecting the presence of particles in each said zone.

13. Apparatus according to any one of the preceding claims, comprising computer means for controlling said electrical input application means and/or for processing signals from said detecting means.

14. A method of testing particles comprising locating the particles in a carrier fluid in a space in different regions of which they are subjected to a plurality of different dielectrophoretic fields and detecting the presence of the particles in the respective regions in order to characterise or identify the particles detected.

15. A method according to claim 14 in which the particles are carried in a fluid that is circulated through said space.

16. A method according to claim 14 or claim 15 wherein the presence of the particles is detected by

measuring the transmission of electromagnetic energy through the respective regions.

17. A method according to claim 14 or claim 15 in which the fields are generated employing a series of spaced electrodes and electrical measurement means are connected to the electrodes to determine the presence of the particles in the fields adjacent the respective electrodes.

18. A method according to any one of claims 14 to 17 wherein a parameter of the fluid carrying the particles is varied to test for any variation of response obtained thereby.

19. A method according to claim 18 wherein said parameter is varied over a period of time while detecting any particles present.

20. A method according to claim 18 wherein said space comprises a plurality of zones in which said fluid parameter is different from zone to zone and particles in the fluid in each zone are subjected to a plurality of different dielectrophoretic fields for detection of particles in the respective zones.

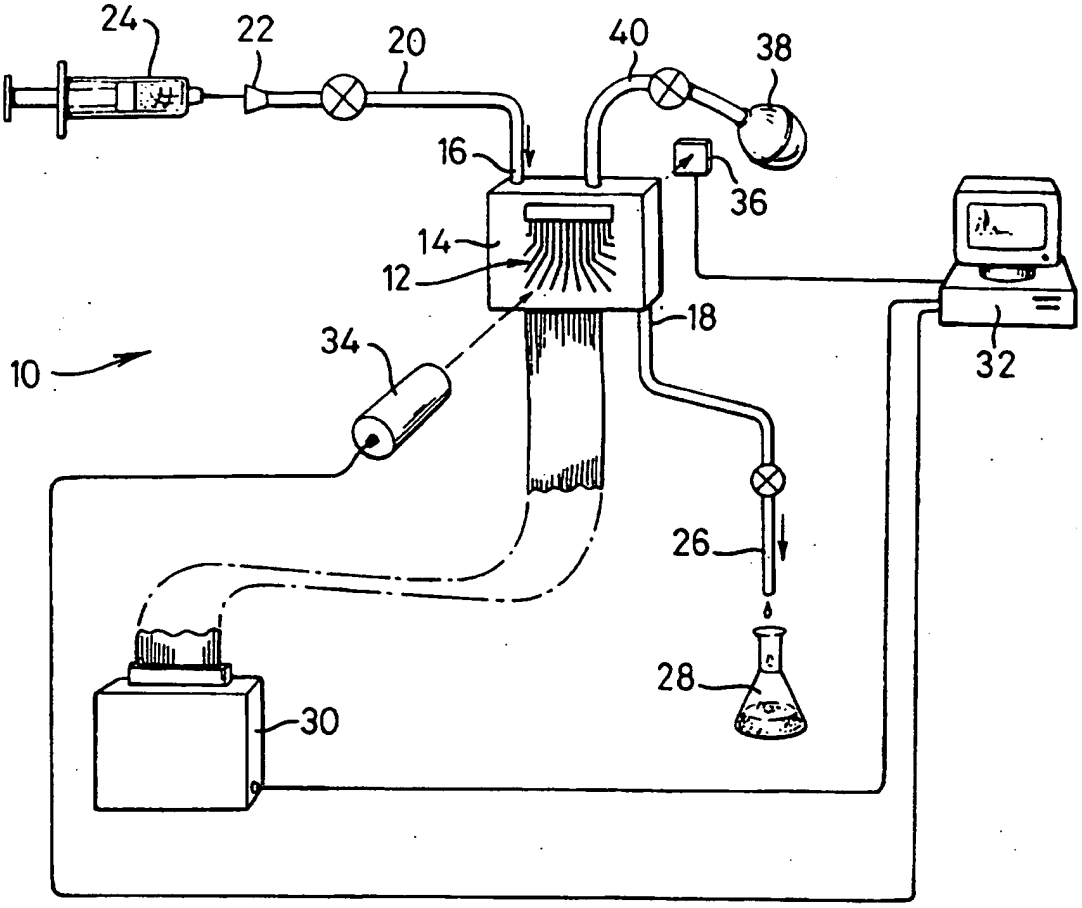



Fig. 1

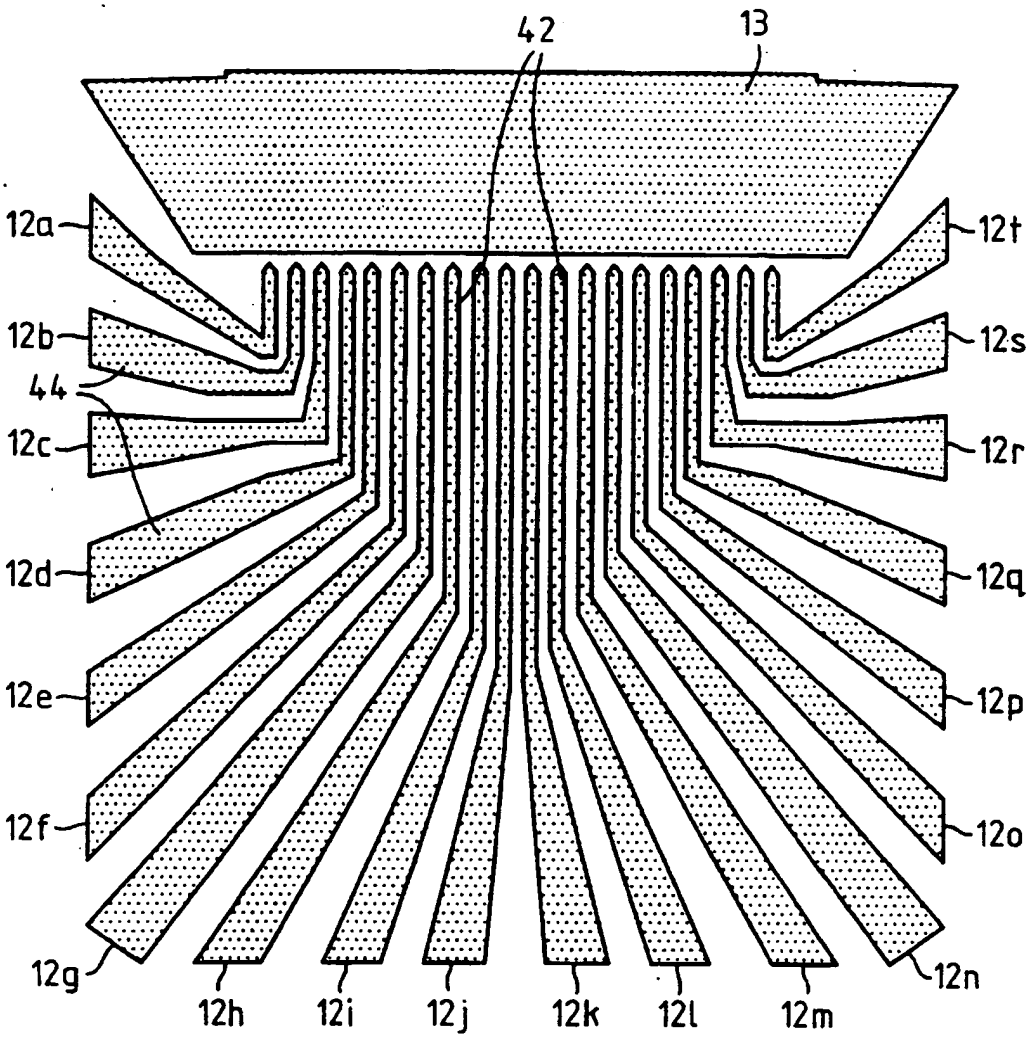

42
13
12a
12t
12b
12s
44
12c
12r
12d
12q
12e
12p
12f
12o
12g
12n
12h
12i
12j
12k
12l
12m

Fig. 2

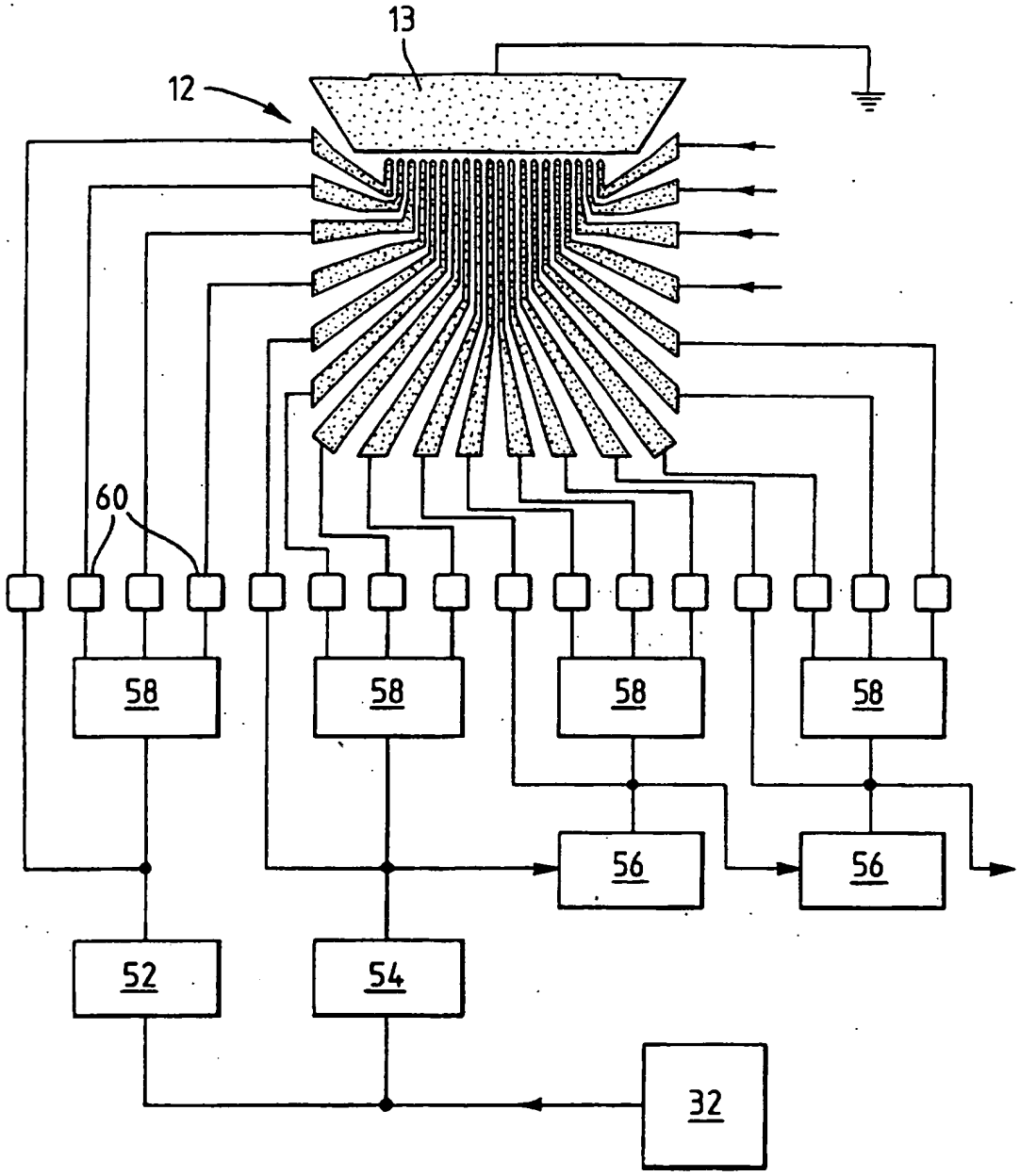



Fig. 3

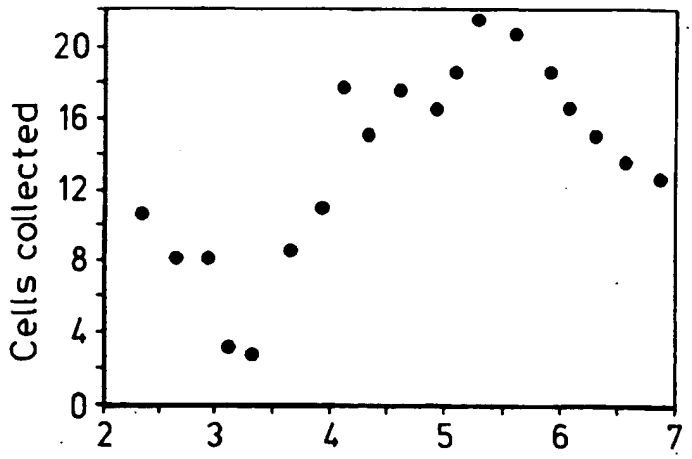



Fig. 4a

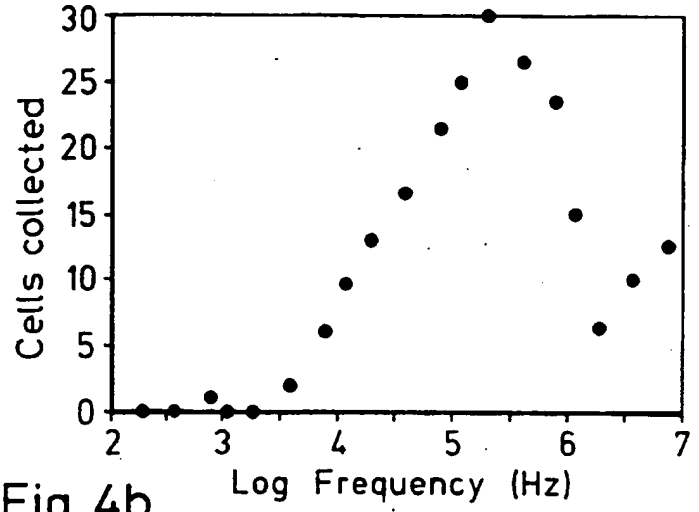



Fig. 4b

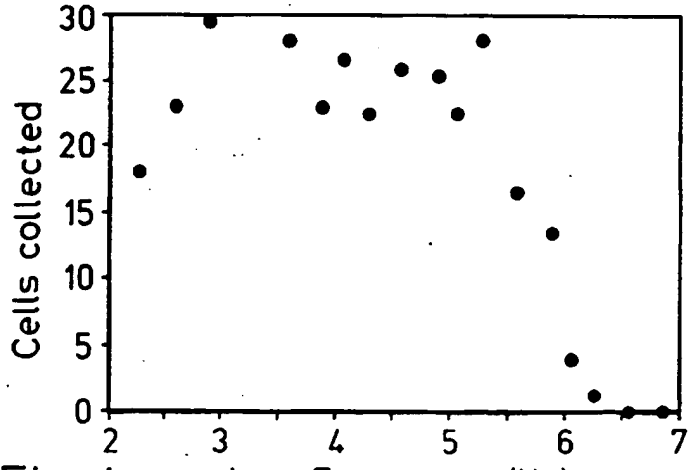



Fig. 4c

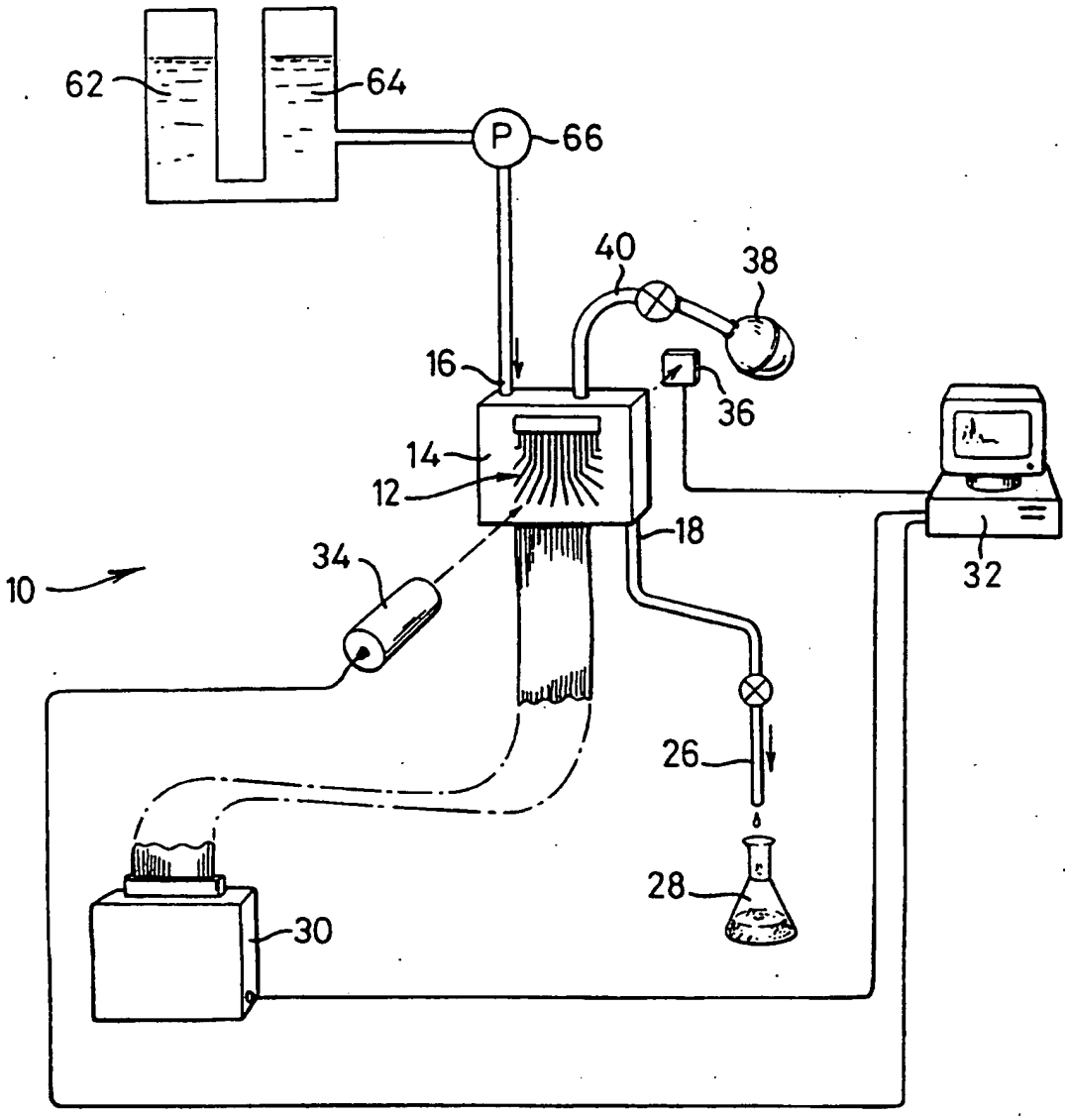



Fig. 5

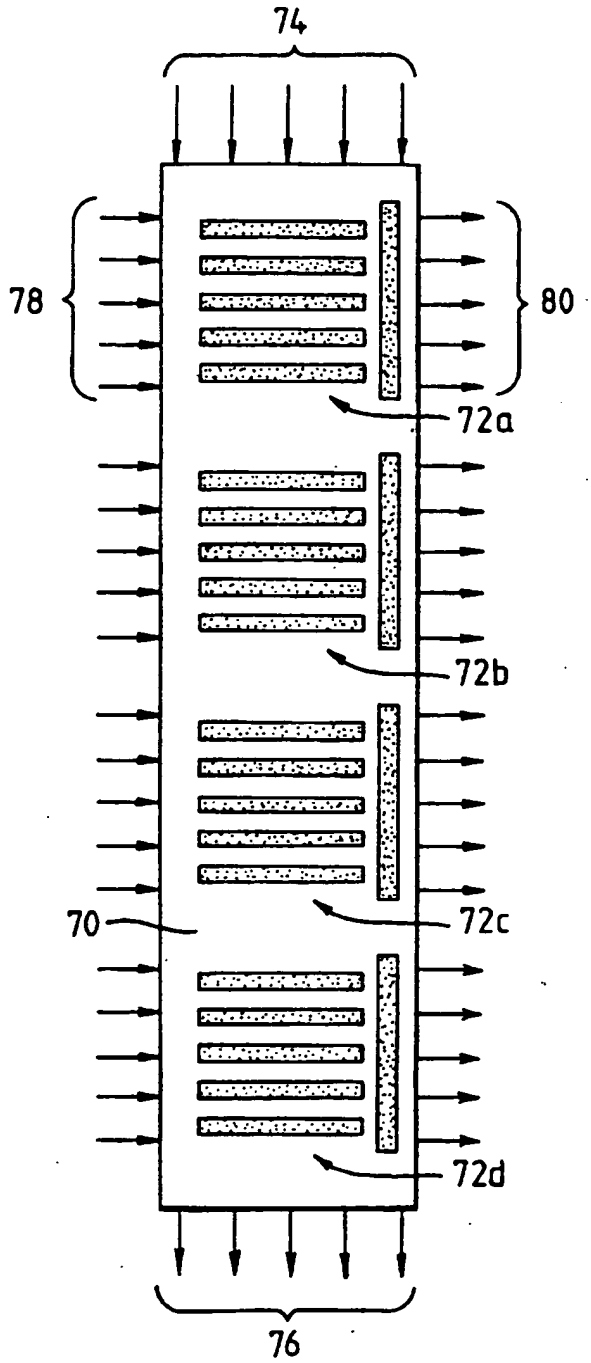



Fig. 6

# INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/GB 97/02011

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B03C5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B03C G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | DE 41 27 405 A (FRAUNHOFER-GESELLSCHAFT ZUR FÖRDERUNG DER ANGEWANDTEN FORSCHUNG EV) 25 February 1993<br>see column 6, line 46 - line 63; figure 4<br>--- | 1 |
| A | J. A. R. PRICE: "APPLICATIONS OF A NEW OPTICAL TECHNIQUE FOR MEASURING THE DIELECTROPHORETIC BEHAVIOUR OF MICRO-ORGANISMS"<br>BIOCHIMICA ET BIOPHYSICA ACTA,<br>vol. 964, 1988,<br>pages 221-230, XP002043977<br>see figure 1<br>---<br>-/-- | 1 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 October 1997 | 28. 10. 97 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Duchatellier, M |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inter. ..onal Application No
PCT/GB 97/02011

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | J. P. H. BURT: "AN OPTICAL DIELECTROPHORESIS SPECTROMETER FOR LOW-FREQUENCY MEASUREMENTS ON COLLOIDAL SUSPENSIONS" JOURNAL OF PHYSICS E: SCIENTIFIC INSTRUMENTS, vol. 22, no. 11, November 1989, BRISTOL, GB, pages 952-957, XP000070410 see abstract; figure 1 --- | 1 |
| A | WO 91 11262 A (P & B (SCIENCES) LTD.) 8 August 1991 see abstract --- | 1 |
| A | WO 92 07657 A (FRAUNHOFER GESELLSCHAFT ZUT FÖRDERUNG DER ANGEWANDTEN FORSCHUNG E.V.) 14 May 1992 see abstract ----- | 1 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten ..onal Application No
PCT/GB 97/02011

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| DE 4127405 A | 25-02-93 | DE 4143573 C | 04-07-96 |
| | | WO 9303850 A | 04-03-93 |
| | | DE 59207522 D | 19-12-96 |
| | | EP 0599957 A | 08-06-94 |
| | | EP 0718038 A | 26-06-96 |
| | | JP 6509745 T | 02-11-94 |
| | | US 5454472 A | 03-10-95 |
| WO 9111262 A | 08-08-91 | AT 146383 T | 15-01-97 |
| | | AU 657086 B | 02-03-95 |
| | | AU 7151191 A | 21-08-91 |
| | | CA 2075042 A | 31-07-91 |
| | | DE 69123726 D | 30-01-97 |
| | | DE 69123726 T | 10-04-97 |
| | | EP 0513064 A | 19-11-92 |
| | | ES 2096644 T | 16-03-97 |
| WO 9207657 A | 14-05-92 | DE 4034697 A | 14-05-92 |
| | | DE 59107393 D | 21-03-96 |
| | | EP 0555252 A | 18-08-93 |
| | | JP 6501159 T | 10-02-94 |